FILE 'REGISTRY' ENTERED AT 15:06:17 ON 10 OCT 2002

=> S POLYETHYLENE GLYCOL/CN
L1 1 POLYETHYLENE GLYCOL/CN

=> S URICASE/CN
L2 1 URICASE/CN

=> D L1;D L2

L1 ANSWER 1 OF 1 REGISTRY COPYRIGHT 2002 ACS
RN 25322-68-3 REGISTRY
CN Poly(oxy-1,2-ethanediyl), .alpha.-hydro-.omega.-hydroxy- (9CI) (CA INDEX NAME)
OTHER NAMES:
CN .alpha.,.omega.-Hydroxypoly(ethylene oxide)
CN .alpha.-Hydro-.omega.-hydroxypoly(oxy-1,2-ethanediyl)
CN .alpha.-Hydro-.omega.-hydroxypoly(oxyethylene)
CN 1,2-Ethanediol, homopolymer
CN 1660O
CN 1660S
CN Alkox
CN Alkox E 100
CN Alkox E 130
CN Alkox E 160
CN Alkox E 240
CN Alkox E 30
CN Alkox E 45
CN Alkox E 60
CN Alkox E 75
CN Alkox R 1000
CN Alkox R 15
CN Alkox R 150
CN Alkox R 400
CN Alkox SR
CN Antarox E 4000
CN Aquacide III
CN Aquaffin
CN Badimol
CN BDH 301
CN Bradsyn PEG
CN Breox 2000
CN Breox 20M
CN Breox 4000
CN Breox 550
CN Breox PEG 300
CN CAFO 154
CN Carbowax
CN Carbowax 100
CN Carbowax 1000
CN Carbowax 1350
CN Carbowax 14000
CN Carbowax 1500
CN Carbowax 1540
CN Carbowax 20
CN Carbowax 200
CN Carbowax 20000
CN Carbowax 25000
CN Carbowax 300
CN Carbowax 3350
CN Carbowax 400
CN Carbowax 4000
CN Carbowax 4500
CN Carbowax 4600
CN Carbowax 600
CN ***Polyethylene glycol***
ADDITIONAL NAMES NOT AVAILABLE IN THIS FORMAT - Use FCN, FIDE, or ALL for DISPLAY
AR 9002-90-8
DR 12676-74-3, 12770-93-3, 9081-95-2, 9085-02-3, 9085-03-4, 54510-95-1, 125223-68-9, 54847-64-2, 59763-40-5, 64441-68-5, 64640-28-4, 133573-31-6,

25104-58-9, 25609-81-8, 134919-43-0, 101677-86-5, 99264-61-6, 106186-24-7,
112895-21-3, 114323-93-2, 50809-04-6, 50809-59-1, 119219-06-6, 60894-12-4,
61840-14-0, 37361-15-2, 112384-37-9, 70926-57-7, 75285-02-8, 75285-03-9,
77986-38-0, 150872-82-5, 154394-38-4, 79964-26-4, 80341-53-3, 85399-22-0,
85945-29-5, 88747-22-2, 34802-42-1, 107502-63-6, 107529-96-4, 116549-90-7,
156948-19-5, 169046-53-1, 188364-77-4, 188924-03-0, 189154-62-9,
191743-71-2, 201163-43-1, 206357-86-0, 221638-71-7, 225502-44-3,
270910-26-4, 307928-07-0, 356055-70-4, 391229-98-4
MF (C2 H4 O)n H2 O
CI PMS, COM
PCT Polyether
LC STN Files: ADISNEWS, AGRICOLA, ANABSTR, AQUIRE, BIOBUSINESS, BIOSIS,
BIOTECHNO, CA, CABA, CANCERLIT, CAPLUS, CASREACT, CBNB, CEN, CHEMCATS,
CHEMINFORMRX, CHEMLIST, CHEMSAFE, CIN, CSCHEM, CSNB, DDFU, DETHERM*,
DIOGENES, DRUGU, EMBASE, ENCOMPLIT, ENCOMPLIT2, ENCOMPPAT, ENCOMPPAT2,
HSDB*, IFICDB, IFIPAT, IFIUDB, IPA, MEDLINE, MRCK*, MSDS-OHS, NIOSHTIC,
PDLCOM*, PIRA, PROMT, RTECS*, SPECINFO, TOXCENTER, TULSA, ULIDAT, USAN,
USPAT2, USPATFULL, VETU, VTB
(*File contains numerically searchable property data)
Other Sources: DSL**, TSCA**, WHO
(**Enter CHEMLIST File for up-to-date regulatory information)

/ Structure 1 in file .gra /

64695 REFERENCES IN FILE CA (1962 TO DATE)
16880 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
64874 REFERENCES IN FILE CAPLUS (1962 TO DATE)

L2 ANSWER 1 OF 1 REGISTRY COPYRIGHT 2002 ACS
RN 9002-12-4 REGISTRY
CN Oxidase, urate (9CI) (CA INDEX NAME)
OTHER NAMES:
CN E.C. 1.7.3.3
CN Urate oxidase
CN Urate: O2-oxidoreductase
CN Uratoxidase
CN Uric acid oxidase
CN ***Uricase***
CN Uricozyme
MF Unspecified
CI MAN
LC STN Files: ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, BIOBUSINESS,
BIOSIS, BIOTECHNO, CA, CAPLUS, CBNB, CHEMCATS, CHEMLIST, CIN, CSCHEM,
DDFU, DRUGU, EMBASE, IFICDB, IFIPAT, IFIUDB, IPA, MEDLINE, MRCK*, PHAR,
PROMT, RTECS*, TOXCENTER, USPAT2, USPATFULL
(*File contains numerically searchable property data)
Other Sources: EINECS**, TSCA**
(**Enter CHEMLIST File for up-to-date regulatory information)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
1467 REFERENCES IN FILE CA (1962 TO DATE)
67 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
1469 REFERENCES IN FILE CAPLUS (1962 TO DATE)

=> S MONOMETHOXYPOLYETHYLENE GLYCOL/CN
L3 1 MONOMETHOXYPOLYETHYLENE GLYCOL/CN

=> D

L3 ANSWER 1 OF 1 REGISTRY COPYRIGHT 2002 ACS
RN 9004-74-4 REGISTRY
CN Poly(oxy-1,2-ethanediyl), .alpha.-methyl-.omega.-hydroxy- (9CI) (CA INDEX
NAME)
OTHER CA INDEX NAMES:
CN Glycols, polyethylene, monomethyl ether (8CI)
OTHER NAMES:

CN  .alpha.-Methyl-.omega.-hydroxypoly(oxy-1,2-ethanediyl)
CN  Breox MPEG 550
CN  Carbowax 2000
CN  Carbowax 350
CN  Carbowax 5000
CN  Carbowax 550
CN  Carbowax 750
CN  Carbowax 750ME
CN  Carbowax MPEG 450
CN  Carbowax MPEG 5000
CN  CP 2000
CN  CP 2000 (polyoxyalkylene)
CN  Ethylene oxide-methanol adduct
CN  GN 8384
CN  Hymol PM
CN  Methoxypoly(ethylene glycol)
CN  Methyl polyglycol
CN  Monomethoxy poly(ethylene oxide)
CN  ***Monomethoxypolyethylene glycol***
CN  Monomethoxypolyoxyethylene
CN  MPEG
CN  MPEG 10000
CN  MPEG 2000
CN  MPEG 350
CN  MPEG 500
CN  MPEG 5000
CN  MPEG 950
CN  MPG
CN  MPG 025
CN  MPG 081
CN  MPG 130
CN  MPG 140
CN  Nissan Uniol 1000
CN  Nissan Uniol 550
CN  Nissan Uniox M 1000
CN  Nissan Uniox M 2000
CN  Nissan Uniox M 400
CN  Nissan Uniox M 4000
CN  Nissan Uniox M 550
CN  Nissan Uniox M 600
CN  O-Methoxypolyethylene glycol
CN  PEGMME
CN  Pluriol A 2000
CN  Pluriol A 2000E
CN  Pluriol A 275E
CN  Pluriol A 350E
CN  Pluriol A 500E
CN  Poly(ethylene oxide) monomethyl ether
CN  Polyethylene glycol methyl ether
ADDITIONAL NAMES NOT AVAILABLE IN THIS FORMAT - Use FCN, FIDE, or ALL for DISPLAY
AR  251911-64-5
DR  165338-17-0, 12623-96-0, 163294-10-8, 163733-28-6, 162582-19-6, 166441-82-3, 158360-78-2, 126966-17-4, 54386-07-1, 57244-93-6, 64543-87-9, 134919-42-9, 95507-78-1, 95507-80-5, 102868-77-9, 104841-59-0, 138753-86-3, 69592-91-2, 72664-19-8, 77102-87-5, 142172-77-8, 146162-92-7, 154701-70-9, 154885-26-4, 86002-19-9, 91826-72-1, 41396-14-9, 178613-33-7, 185250-24-2, 187523-66-6, 189209-93-6, 193008-24-1, 195970-98-0, 207799-14-2, 212969-32-9, 216693-45-7, 226212-72-2, 237739-71-8, 241466-57-9, 396134-26-2
MF  (C2 H4 O)n C H4 O
CI  PMS, COM
PCT Polyether
LC  STN Files:  AGRICOLA, BIOBUSINESS, BIOSIS, BIOTECHNO, CA, CANCERLIT, CAPLUS, CASREACT, CEN, CHEMCATS, CHEMINFORMRX, CHEMLIST, CIN, CSCHEM, DETHERM*, EMBASE, IFICDB, IFIPAT, IFIUDB, IPA, MEDLINE, MSDS-OHS, NIOSHTIC, PIRA, PROMT, RTECS*, TOXCENTER, USAN, USPAT2, USPATFULL, VTB
(*File contains numerically searchable property data)
Other Sources:  DSL**, TSCA**
(**Enter CHEMLIST File for up-to-date regulatory information)

/ Structure 2 in file .gra /

2599 REFERENCES IN FILE CA (1962 TO DATE)
1018 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
2613 REFERENCES IN FILE CAPLUS (1962 TO DATE)

FILE 'CAPLUS' ENTERED AT 15:09:17 ON 10 OCT 2002

=> S L1;S L2;S L3
L4 65050 L1

L5 1469 L2

L6 2616 L3

=> S PEG
23847 PEG
867 PEGS
L7 24230 PEG
(PEG OR PEGS)

=> S LYSINE;S URICASE
87291 LYSINE
1739 LYSINES
L8 87820 LYSINE
(LYSINE OR LYSINES)

1873 URICASE
27 URICASES
L9 1877 URICASE
(URICASE OR URICASES)

=> S (L4,L6) AND L8;S (L4,L6) AND (L9 OR L2)
L10 477 ((L4 OR L6)) AND L8

1469 L2
L11 47 ((L4 OR L6)) AND (L9 OR L2)

=> S L2
L12 1469 L2

=> S (L4,L6) AND L8;S (L4,L6) AND (L9 OR L12)
L13 477 ((L4 OR L6)) AND L8

L14 47 ((L4 OR L6)) AND (L9 OR L12)

=> S (L4,L6) AND L8 AND (L9,L12)
L15 1 ((L4 OR L6)) AND L8 AND ((L9 OR L12))

=> D CBIB ABS

L15 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2002 ACS
2002:107165 Document No. 136:172754 Highly reactive branched polymer and proteins or peptides conjugated with the polymer. Park, Myung-Ok; Lee, Kang-Choon; Cho, Sung-hHe (S. Korea). PCT Int. Appl. WO 2002009766 A1 20020207, 47 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-KR1209 20010713. PRIORITY: KR 2000-44046 20000729.
AB The present invention relates to new biocompatible polymer derivs., and a

protein-polymer or a peptide-polymer which is produced by conjugation of biol. active protein and peptide with the biocompatible polymer derivs. More particularly, the present invention relates to a highly reactive branched biocompatible polymer deriv. contg. a long linker between polymer derivs. and protein or peptide mols., which is minimized in decrease the biol. activity of proteins by conjugating the less no. of polymer derivs. to the active sites of proteins, improved in water soly., and protected from being degraded by protease. In hence, the highly reactive branched biocompatible polymer-proteins or peptides conjugates with long linker retain the biol. activity for a long period of time and improve a bioavailability of bioactive proteins and peptides. For example, activated PEG-interferon conjugates were prepd. by adding 3 mg of succinic N-hydroxysuccinimidyl di-PEG to 3 mg of interferon in 0.1 M phosphate buffer soln., pH 7.0 at ambient temp. The reaction was stopped with 0.1 M glycine and the excess reagents were using Centricon-30.

```
=> S L13 AND L14
L16          1 L13 AND L14

=> S L13 OR L14
L17        523 L13 OR L14

=> S L17 AND PD<1998
      18116477 PD<1998
                  (PD<19980000)
L18        276 L17 AND PD<1998

=> S ACTIVITY
       1793897 ACTIVITY
        348863 ACTIVITIES
L19    1935955 ACTIVITY
                  (ACTIVITY OR ACTIVITIES)

=> S L19 AND L17
L20        104 L19 AND L17

=> S L19 (10A) L8;S L19 (10A) L7
L21       3651 L19 (10A) L8


L22        792 L19 (10A) L7

=> S L17 AND L21;S L17 AND L22
L23          8 L17 AND L21


L24         24 L17 AND L22

=> S L23,L24
L25         27 (L23 OR L24)

=> D 1-27 CBIB ABS
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L25 ANSWER 1 OF 27 CAPLUS COPYRIGHT 2002 ACS

2002:352709 Document No. 137:105575 Coupling reaction and properties of poly(ethylene glycol)-linked phospholipases A2. Bianco, Ismael D.; Daniele, Jose J.; Delgado, Cristina; Fisher, Derek; Francis, Gillian E.; Fidelio, Gerardo D. (CEPROCOR-Agencia Cordoba Ciencia S.E., Cordoba, Argent.). Bioscience, Biotechnology, and Biochemistry, 66(4), 722-729 (English) 2002. CODEN: BBBIEJ. ISSN: 0916-8451. Publisher: Japan Society for Bioscience, Biotechnology, and Agrochemistry.

AB Secretory phospholipases A2 (PLA2) from Naja naja naja (cobra snake) venom, from Bothrops neuwiedii (crotalid snake) venom (two isoforms) and from bee venom were modified with tresylated monomethoxy poly(ethylene glycol) (TMPEG). The kinetic and inflammatory properties of the adducts (PEG-PLA2) were measured. As found by gel permeation chromatog., 95-100% of P-1 PLA2 from B. neuwiedii and PLA2 from N. naja naja venom change their chromatog. mobility after TMPEG treatment. By contrast, only 50-60% of both P-3-PLA2 from B. neuwiedii and PLA2 from bee venom modify their elution profile from Superdex 75. All the modified proteins preserved the

enzymic activity toward phospholipid monolayers, but with a reduced specific activity and greater lag times than the unmodified controls. These results suggest that the PEG-PLA2 complexes would have an altered interaction with lipid membranes. The ***PEG*** -linked proteins preserve their edema-inducing ***activity*** evaluated by the rat hind-paw edema test except for N. naja naja ***PEG*** -PLA2 in which inflammatory ***activity*** was significatively decreased. Altogether, the results show a partial dissocn. of catalytic and inflammatory activities of Group II and III secretory PLA2s after their modification with PEG.

L25 ANSWER 2 OF 27 CAPLUS COPYRIGHT 2002 ACS

2001:598143 Document No. 135:185437 Aggregate-free urate oxidase for preparation of non-immunogenic polymer conjugates with increased serum persistence. Sherman, Merry R.; Saifer, Mark G. P.; Williams, L. David (Mountain View Pharmaceuticals, Inc., USA). PCT Int. Appl. WO 2001059078 A2 20010816, 23 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, CZ, DE, DE, DK, DK, DM, DZ, EE, EE, ES, FI, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US40069 20010207. PRIORITY: US 2000-501730 20000210.

AB The invention relates to removal of aggregates larger than octamers from urate oxidases ( ***uricases*** ) prior to conjugation of poly(ethylene glycols) or poly(ethylene oxides). This substantially eliminates ***uricase*** immunogenicity without compromising its uricolytic activity. Preparative ion-exchange chromatog. of ***uricase*** , size-exclusion chromatog. of ***uricase*** monitored by light scattering and UV absorbance, and synthesis of PEG- ***uricase*** conjugates, are described. In vivo serum persistence and immunogenicity of ***uricase*** and PEG- ***uricase*** are studied. Uricolytic ***activity*** ELISA assays of ***PEG*** - ***uricase*** in sera from mice injected with PEG- ***uricase*** are performed. A naturally occurring or recombinant protein, esp. a mutein of porcine urate oxidase ( ***uricase*** ), that is essentially free of large aggregates can be rendered substantially non-immunogenic by conjugation with a sufficiently small no. of strands of polymer such that the bioactivity of the protein is essentially retained in the conjugate. Such conjugates are unusually well suited for treatment of chronic conditions because they are less likely to induce the formation of antibodies and/or accelerated clearance than are similar conjugates prepd. from protein prepns. contg. traces of large aggregates.

L25 ANSWER 3 OF 27 CAPLUS COPYRIGHT 2002 ACS

2000:530198 Document No. 133:227663 Therapeutic proteins: a comparison of chemical and biological properties of ***uricase*** conjugated to linear or branched poly(ethylene glycol) and poly(N-acryloylmorpholine). Schiavon, O.; Caliceti, P.; Ferruti, P.; Veronese, F. M. (Department of Pharmaceutical Sciences, Center of Chemical Investigation of Drugs CNR, University of Padua, Padua, Italy). Farmaco, 55(4), 264-269 (English) 2000. CODEN: FRMCE8. ISSN: 0014-827X. Publisher: Elsevier Science S.A..

AB ***Uricase*** from Bacillus fastidiosus (UC) was covalently linked to linear PEG (PEG-1) (Mw 5 kDa), branched PEG (PEG-2) (Mw 10 kDa) and to poly(N-acryloylmorpholine) (PAcM) (Mw 6 kDa). The conjugation of UC with linear ***PEG*** and PAcM was accompanied by complete loss of enzymic ***activity*** but, if uric acid as site protecting agent was included in the reaction mixt., the conjugate protein retained enzymic activity. On the other hand, the modification with ***PEG*** -2 gave a conjugate that also maintained enzymic ***activity*** in the absence of any active site protection. This behavior must be related to hindrance of the branched polymer in reaching the enzyme active site. The UC conjugates exhibited increased resistance to proteolytic digestion while minor variations in the inhibitory const., optimal pH, heat stability, affinity for substrate, were obsd. Pharmacokinetic investigations in mice demonstrated increased residence time in blood for all the conjugates as compared with native ***uricase*** . ***Uricase*** conjugated with linear PEG was longer lasting in blood UC deriv., followed by branched PEG

and the PAcM conjugates. Unconjugated ***uricase*** was rapidly removed from circulation. All these data are in favor of the use of the less known amphiphilic polymer PAcM as an alternative to PEGs in modification of enzymes devised for therapeutic applications.

L25 ANSWER 4 OF 27 CAPLUS COPYRIGHT 2002 ACS
2000:125571 Document No. 132:269962 Tumor-targeting chemotherapy by a xanthine oxidase-polymer conjugate that generates oxygen-free radicals in tumor tissue. Sawa, Tomohiro; Wu, Jun; Akaike, Takaaki; Maeda, Hiroshi (Department of Microbiology, Kumamoto University School of Medicine, Kumamoto, 860-0811, Japan). Cancer Research, 60(3), 666-671 (English) 2000. CODEN: CNREA8. ISSN: 0008-5472. Publisher: AACR Subscription Office.

AB Xanthine oxidase (XO) mediates anticancer activity because of its ability to generate cytotoxic reactive oxygen species (ROS), including superoxide anion radical and hydrogen peroxide. However, the high binding affinity of XO to blood vessels would cause systemic vascular damage and hence limits the use of native XO in clin. settings. The authors demonstrate here that chem. conjugation of XO with poly(ethylene glycol) (PEG; the conjugates hereafter referred to as ***PEG*** -XO) significantly enhanced the tumor-targeting efficacy and the antitumor ***activity*** of XO. By using a succinimide-activated PEG deriv., PEG was conjugated to .epsilon.-amino groups of ***lysine*** residues of XO, which play a crucial role in binding of XO to blood vessels. PEG-XO administered i.v. showed a 2.8-fold higher accumulation in solid tumor compared with that of native XO 24 h after injection, whereas a slight or negligible increase in accumulation of PEG-XO was obsd. in normal organs. The highest ***PEG*** -XO enzyme ***activity*** was detected in tumor compared with normal organs or tissues except blood; enzyme activity in tumor was 5.0, 3.9, and 9.4 times higher than that in liver, kidney, and spleen, resp. Intratumor activity remained high for >48 h. Administration of hypoxanthine, a substrate of XO, at 33 mg/kg body wt. i.p. 12 h after the administration of PEG-XO (0.6 unit/mouse, i.v.) resulted in significant suppression of tumor growth ($P<0.001$), with no tumor growth even after 52 days. However, either PEG-XO or hypoxanthine alone, or native XO with hypoxanthine, showed no effect on the inhibition of tumor growth under present exptl. conditions. These findings suggest that PEG-XO, which accumulates preferentially in tumor tissue, warrants further investigation as a novel anticancer agent.

L25 ANSWER 5 OF 27 CAPLUS COPYRIGHT 2002 ACS
1999:723904 Document No. 131:332732 Folate Copolymer-Mediated Transfection of Cultured Cells. Leamon, Christopher P.; Weigl, Debra; Hendren, R. Wayne (Department of Oligomer Development, GlaxoWellcome Research Institute, Research Triangle Park, NC, 27709, USA). Bioconjugate Chemistry, 10(6), 947-957 (English) 1999. CODEN: BCCHES. ISSN: 1043-1802. Publisher: American Chemical Society.

AB Poly(ethylene glycol) of various sizes was used as a mol. spacer to sep. the cell-targeting ligand, folate, from the surface of poly-L-***lysine*** . The resulting ternary macromol. (pLys-PEG-folate) was investigated in various formulations for its ability to transfect reporter plasmids into receptor-bearing HeLa and IGROV cell lines. Formulations were optimized with respect to DNA content, .+-. charge ratio, and the size and amt. of PEG substitution off the pLys backbone. Transfection ***activity*** was highest 48 h after sample introduction, and ***PEG*** 3400 was detd. to be the most favorable spacer size tested. PLys-PEG-folate:DNA transfection was also found to be both concn. dependent and saturable; plus, it was blocked by the addn. of excess-free folate, indicative of a specific mechanism of uptake. And, cell viability remained greater than 85% at the highest concns. of pLys-PEG-folate:DNA complexes tested (4.8 .mu.g/mL pLys 331 000; 12 .mu.g/mL DNA). Taken together, these observations provide evidence that pLys-PEG-folate:DNA complexes are taken up specifically by the folate endocytosis pathway, and that the intramol. spatial distance of the ligand from the pLys backbone dramatically influences transfection.

L25 ANSWER 6 OF 27 CAPLUS COPYRIGHT 2002 ACS
1998:432253 Document No. 129:207092 Brain-derived neurotrophic factor (BDNF). Sakane, Toshiyasu; Pardridge, William M. (Fac. Pharm. Sci., Setsunan Univ., Japan). Drug Delivery System, 13(3), 173-178 (Japanese) 1998. CODEN: DDSYEI. ISSN: 0913-5006. Publisher: Nippon DDS Gakkai

Jimukyoku.

AB Brain-derived neurotrophic factor (BDNF) was modified by carboxyl-directed protein pegylation in order to retain biol. activity and reduce the systemic clearance of this cationic protein in vivo. Since the modification of the surface ***lysine*** residue of BDNF resulted in loss of biol. ***activity*** , the present study examine the feasibility of placing polyethylene glycol ( ***PEG*** ) polymer on the carboxyl residue of BDNF. PEG mols. with terminal hydrazide moiety of mol. wt. 2 kDa (PEG2000-Hz) and 5 kDa (PEG5000-Hz) were coupled to BDNF carboxyls using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide. Polyacrylamide gel electrophoresis revealed that the mol. wt. distributions of BDNF-PEG2000 and BDNF-PEG5000 were 20 K.apprx.30 kDa and 60 k.apprx.80 kDa, resp. Cell survival studies using 3T3 cells transfected with the BCNF receptor gene showed that the biol. activity of BDNF was not changed following pegylation with PEG2000, and was minimally impaired following pegylation with PEG5000. The systemic clearances of the 125I-BDNF-PEG2000 and 125I-BDNF-PEG5000 following i.v. administration to rats were reduced 67% and 91%, resp., compared to unconjugated BDNF. TCA sol. fraction of the radioactivity in the plasma 60 min following administration were reduced 72% and 94%, resp., showing the significant redn. of the BDNF metab. by pegylation. These expts. describe the first successful carboxyl-directied pegylation of a neuropeptide, and show that this formulation substantially reduces the systemic clearance and metab. of this neurotrophic factor. In conclusion, the carboxyl-directed pegylation is beneficial to the modification of the protein whose surface ***lysine*** or arginine residue is essential to the biol. ***activity*** .

L25 ANSWER 7 OF 27 CAPLUS COPYRIGHT 2002 ACS

1997:573078 Document No. 127:243605 Carboxyl-directed pegylation of brain-derived neurotrophic factor markedly reduces systemic clearance with minimal loss of biologic activity. Sakane, Toshiyasu; Pardridge, William M. (Department of Medicine, UCLA School of Medicine, Los Angeles, CA, 90095-1682, USA). Pharmaceutical Research, 14(8), 1085-1091 (English) 1997. CODEN: PHREEB. ISSN: 0724-8741. Publisher: Plenum.

AB Brain-derived neurotrophic factor (BDNF) was modified by carboxyl-directed protein pegylation in order to both retain biol. activity of the neurotrophin and reduce the rate of systemic clearance of this cationic protein in vivo. Since the modification of surface ***lysine*** residues of neurotrophins results in loss of biol. ***activity*** , the present studies examine the feasibility of placing polyethyleneglycol ( ***PEG*** ) polymers on carboxyl residues of surface glutamate or aspartate residues of BDNF. PEG mols. with terminal hydrazide (Hz) moieties of mol. wt. 2000 (PEG2000-Hz) or 5000 (PEG5000-Hz) daltons were coupled to BDNF carboxyls using carbodiimide. The systemic clearances of the BDNF-PEG2000 and BDNF-PEG5000 were reduced 67% and 91%, resp., compared to unconjugated BDNF. The brain vol. of distribution (VD) of BDNF-PEG5000 was not significantly different from the cerebral plasma vol. Cell survival studies and TrkB auto-phosphorylation assays showed that the biol. activity of BDNF was not changed following pegylation with PEG2000, and was minimally impaired following pegylation with PEG5000. These expts. describe the first carboxyl-directed pegylation of a neuropeptide, and show this formulation substantially reduces the systemic distribution and elimination of the neurotrophic factor. The biol. activity of the neurotrophin is retained with carboxyl-directed pegylation.

L25 ANSWER 8 OF 27 CAPLUS COPYRIGHT 2002 ACS

1997:81096 Document No. 126:156272 Structure-function studies of interleukin 15 using site-specific mutagenesis, polyethylene glycol conjugation, and homology modeling. Pettit, Dean K.; Bonnert, Timothy P.; Eisenman, June; Srinivasan, Subhashini; Paxton, Ray; Beers, Courtney; Lynch, Dave; Miller, Bob; Yost, Jeff; Grabstein, Kenneth H.; Gombotz, Wayne R. (Departments Analytical Chemistry and Formulation, Immunex Corporation, Seattle, WA, 98101, USA). Journal of Biological Chemistry, 272(4), 2312-2318 (English) 1997. CODEN: JBCHA3. ISSN: 0021-9258. Publisher: American Society for Biochemistry and Molecular Biology.

AB Interleukin (IL)-15 is a multifunctional cytokine that shares many biol. activities with IL-2. This functional overlap, as well as receptor binding subunits shared by IL-15 and IL-2, suggests tertiary structural similarities between these two cytokines. In this study, recombinant human IL-15 was PEGylated via ***lysine*** -specific conjugation chem.

to extend the circulation half-life of this cytokine. Although PEGylation did extend the .beta.-elimination circulation half-life of IL-15 by greater than 50-fold, the biol. ***activity*** of polyethylene glycol ( ***PEG*** )-IL-15 was significantly altered. Specifically, PEG-IL-15 lost its ability to stimulate the proliferation of CTLL but took on the properties of a specific IL-15 antagonist in vitro. In comparing sequence alignments and mol. models for IL-2 and IL-15, it was noted that ***lysine*** residues resided in regions of IL-15 that may have selectively disrupted receptor subunit binding. The authors hypothesized that PEGylation of IL-15 interferes with .beta. but not .alpha. receptor subunit binding, resulting in the IL-15 antagonist activity obsd. in vitro. The validity of this hypothesis was tested by engineering site-specific mutants of human IL-15 as suggested by the IL-15 model (IL-15D8S and IL-15Q108S block .beta. and .gamma. receptor subunit binding, resp.). As with PEG-IL-15, these mutants were unable to stimulate CTLL proliferation but were able to specifically inhibit the proliferation of CTLL in response to unmodified IL-15. These results supported the authors' model of IL-15 and confirmed that interference of .beta. receptor subunit binding by adjacent PEGylation could be responsible for the altered biol. ***activity*** obsd. for ***PEG*** -IL-15.

L25 ANSWER 9 OF 27 CAPLUS COPYRIGHT 2002 ACS

1996:600015 Document No. 125:245357 Molecular design of hybrid tumor necrosis factor-.alpha. III: polyethylene glycol-modified tumor necrosis factor-.alpha. has markedly enhanced antitumor potency due to longer plasma half-life and higher tumor accumulation. Tsutsumi, Y.; Kihara, T.; Tsunoda, S.; Kamada, H.; Nakagawa, S.; Kaneda, Y.; Kanamori, T.; Mayumi, T. (Fac. Pharmaceutical Scis., Osaka Univ., Osaka, 565, Japan). Journal of Pharmacology and Experimental Therapeutics, 278(3), 1006-1011 (English) 1996. CODEN: JPETAB. ISSN: 0022-3565. Publisher: Williams & Wilkins.

AB We have reported that chem. modification of tumor necrosis factor-.alpha. (TNF-.alpha.) with polyethylene glycol (PEG) markedly increases its antitumor potency without any adverse side effects. MPEG-TNF-.alpha., esp., in which 56% of the ***lysine*** amino groups of TNF-.alpha. are coupled with ***PEG*** , exhibits 100-fold more antitumor ***activity*** in vivo than native TNF-.alpha. in the Meth-A murine sarcoma model. In this study, we investigated the pharmacokinetics of PEG-modified TNF-.alpha. with various mol. sizes to clarify the mechanisms of the enhanced antitumor potency of MPEG-TNF-.alpha.. The plasma half-lives of modified TNF-.alpha. increased with increasing mol. size. The decreased plasma clearance of modified TNF-.alpha. was partially caused by the shielding effect of the proteolytic sites in TNF-.alpha. by the attached PEG and the decreased transport from blood to various tissues. Almost all native TNF-.alpha. was uniformly distributed to the kidney and reticuloendothelial system within 1 h of an i.v. administration, and rapidly disappeared from these tissues at 3 h. However, very little native TNF-.alpha. was transported into the tumor. The abs. distributed amt. and distribution profile of modified TNF-.alpha. to tissues other than the tumor were the same as those of native TNF-.alpha., whereas the plasma levels of the modified TNF-.alpha. were higher than plasma levels of the native TNF-.alpha.. The tumor distribution of modified TNF-.alpha. was markedly enhanced compared with native TNF-.alpha. and gradually increased over time. About 9-fold more MPEG-TNF-.alpha. was distributed to the tumor than native TNF-.alpha.. Thus, we found that the marked increase in the antitumor potency of PEG-modified TNF-.alpha. resulted from the enhanced blood residency and tumor accumulation. The antitumor effect of MPEG-TNF-.alpha. against sarcoma-180 other than Meth-A fibrosarcoma was also about 100 times greater than that of native TNF-.alpha. when systematically administered. The optimal PEGylation of TNF-.alpha. facilitated its antitumor potency and MPEG-TNF-.alpha. may be a useful systemic antitumor therapeutic drug.

L25 ANSWER 10 OF 27 CAPLUS COPYRIGHT 2002 ACS

1996:522600 Document No. 125:230444 Molecular design of hybrid cytokines with water soluble polymers. Tsutsumi, Y.; Nakagawa, S.; Mayumi, T. (Faculty and Graduate School of Pharmaceutical Sciences, Osaka University, Suita, 565, Japan). Proceedings of the International Symposium on Controlled Release of Bioactive Materials, 23rd, 871-872 (English) 1996. CODEN: PCRMEY. ISSN: 1022-0178. Publisher: Controlled Release Society, Inc..

AB Natural human tumor necrosis factor-.alpha. (TNF-.alpha.) is conjugated with PEG to increase its stability. The specific ***activity*** of ***PEG*** -TNF-.alpha. (I) relative to that of native TNF-.alpha. gradually decreased with an increase in the degree of PEG modification, but the plasma half-life was drastically increased with the increase in mo. wt. of modified TNF-.alpha.. I, in which 56% of ***lysine*** residues were coupled with PEG, were approx. 100 times more anti-tumor potent than unmodified TNF-.alpha. in murine model.

L25 ANSWER 11 OF 27 CAPLUS COPYRIGHT 2002 ACS
1996:28296 Document No. 124:105778 An Adduct of cis-Diaminodichloroplatinum(II) and Polyethylene glycol-poly(L- ***lysine*** )-Succinate: Synthesis and Cytotoxic Properties. Bogdanov, A. A., Jr.; Martin, C.; Bogdanova, A.; Brady, T. J.; Weissleder, R. (Department of Radiology, Massachusetts General Hospital, Boston, MA, 02129, USA). Bioconjugate Chemistry, 7(1), 144-9 (English) 1996. CODEN: BCCHES. ISSN: 1043-1802. Publisher: American Chemical Society.
AB A noncovalent adduct of the antineoplastic drug cis-diaminodichloroplatinum (cDDP) and a biocompatible graft copolymer of poly(L- ***lysine*** ) and methoxypolyethylene glycol succinate is described. Upon incubation of cDDP with [O-methylpolyethylene glycol-O'-succinyl]-N-.epsilon.-poly(L- ***lysine*** )n-N-.epsilon.-succinate (n = 250-270) highly sol., long circulating adducts were formed which contained 4.3% of platinum by wt. Approx. 60% of the polymer-assocd. drug was released during dialysis against saline or serum albumin contg. saline, with a half-time of release of 63 h. The adducts showed a pronounced antineoplastic effect in BT-20 human adenocarcinoma cell cultures. In cell proliferation assays, the concn. of half-inhibition of [3H]thymidine uptake was 0.9 .+-. 0.2 .mu.M for the drug-copolymer adduct compared to 0.3 .+-. 0.1 .mu.M for free cDDP. The adduct showed a long blood half-life (ca. 14 h in rats) and accumulated in exptl. mammary adenocarcinomas at 2.5-3.5% injected dose per g of tissue. A control adduct of cDDP with the backbone portion of the copolymer, poly(L- ***lysine*** )-N-.epsilon.-succinate, had a short half-life in the bloodstream (ca. 30 min) and low accumulation (0.5% injected dose/g) in tumor. A dual therapeutic effect of methylpoly(ethylene glycol)succinylpoly(L- ***lysine*** )-succinate as a carrier of cDDP is suggested: (1) as a carrier for systemic release of the active drug from the macromol. while it circulates in the bloodstream and (2) as a carrier for on-site delivery which results from the release of the drug in the tumor as a consequence of accumulation of the copolymer in the tumor.

L25 ANSWER 12 OF 27 CAPLUS COPYRIGHT 2002 ACS
1995:753042 Document No. 123:188105 Characterization of ***PEG*** -IL-6 and its thrombopoietic ***activity*** in vivo. Tsunoda, Shin-ichi; Kihira, Tetsunari; Kamada, Haruhiko; Okada, Naoki; Osugi, Yoshiyuki; Tsutsumi, Yasuo; Nakagawa, Shinsaku; Mayumi, Tadanori (Fac. Pharm. Sci., Osaka Univ., Suita, 565, Japan). Drug Delivery System, 10(3), 175-80 (Japanese) 1995. CODEN: DDSYEI. ISSN: 0913-5006. Publisher: Nippon DDS Gakkai Jimukyoku.
AB The chem. modification of human recombinant interleukin-6(IL-6) with polyethylene glycol ( ***PEG*** ) was conducted to increase its thrombopoietic ***activity*** . PEG-modified IL-6 (PEG-IL-6) was sepd. into several mol. wt. fractions and characterized individually. The specific ***activity*** of ***PEG*** -IL-6 was reduced with increase in its degree of PEG-modification, i.e., whole mol. wt. The in vivo thrombopoietic ***activity*** of ***PEG*** -IL-6 was markedly increased. Esp., PEG-IL-6, in which 54% of the 14 ***lysine*** amino groups of IL-6 were coupled with PEG, showed 10 times greater thrombopoietic effect than native IL-6. Pharmacokinetic anal. indicated that PEG-IL-6 greatly enlarged the area under the time-concn. curve. These findings suggested that PEG-IL-6 may be a useful agent for the treatment of thrombocytopenia after chemotherapy.

L25 ANSWER 13 OF 27 CAPLUS COPYRIGHT 2002 ACS
1995:616048 Document No. 123:81299 Molecular design of hybrid tumor necrosis factor alpha with polyethylene glycol increases its anti-tumor potency. Tsutsumi, Y; Kihira, T; Tsunoda, S; Kanamori, T; Nakagawa, S; Mayumi, T (Faculty of Pharmaceutical Sciences, Osaka University, Suita, 565, Japan). British Journal of Cancer, 71(5), 963-8 (English) 1995. CODEN: BJCAAI. ISSN: 0007-0920.

AB This study was conducted to increase the anti-tumor potency and reduce the toxic side-effects of tumor necrosis factor alpha (TNF-.alpha.). Natural human TNF-.alpha. was chem. conjugated with monomethoxy polyethylene glycol (PEG) using succinimidyl coupling of ***lysine*** amino groups of TNF-.alpha.. The no.-av. mol. wt. of PEG-modified TNF-.alpha. (PEG-TNF-.alpha.) increased with an increase in the reaction time and the initial molar ratio of PEG relative to TNF-.alpha.. The resulting modified TNF-.alpha. was sepd. into fractions of various mol. wts. The specific ***activity*** of sepd. ***PEG*** -TNF-.alpha. relative to that of native TNF-.alpha. gradually decreased with an increase in the degree of PEG modification, but the plasma half-life was drastically increased with the increase in mol. wt. of modified TNF-.alpha.. PEG-TNF-.alpha., in which 29% and 56% of ***lysine*** residues were coupled to ***PEG*** , had anti-tumor ***activity*** approx. 4 and 100 times greater than unmodified TNF-.alpha. in the murine Meth-A fibrosarcoma model. Extensive ***PEG*** modification did not increase its in vivo ***activity*** . A high dose of unmodified TNF-.alpha. induced toxic side-effects, but these were not obsd. with the modified TNF-.alpha.. Optimal PEG modification of TNF-.alpha. markedly increased its bioavailability and may facilitate its potential anti-tumor therapeutic use.

L25 ANSWER 14 OF 27 CAPLUS COPYRIGHT 2002 ACS

1994:124256 Document No. 120:124256 Polyethylene glycol-modified chimeric toxin composed of transforming growth factor .alpha. and Pseudomonas exotoxin. Wang, Qing Cheng; Pai, Lee H.; Debinski, Waldemar; FitzGerald, David J.; Pastan, Ira (Div. Cancer Biol., Natl. Cancer Inst., Bethesda, MD, 20892, USA). Cancer Research, 53(19), 4588-94 (English) 1993. CODEN: CNREA8. ISSN: 0008-5472.

AB Modification of proteins with monomethoxy-polyethylene glycol (mPEG) has been shown to prolong circulation time and to reduce immunogenicity. To make a mPEG-modified recombinant toxin that retained cytotoxic activity but had a longer residence time in circulation, the authors have constructed on altered form of TGF.alpha.-PE40, a recombinant toxin composed of human transforming growth factor .alpha. (TGF.alpha.) fused to a fragment of Pseudomonas exotoxin (PE38) devoid of its cell-binding domain. In the newly designed protein, termed TGF.alpha.R29-L2-CH2-PE38QQ.DELTA. (TCP), there are no ***lysine*** residues in the TGF.alpha. and PE38 portions. Human IgG4 const. region CH2 and a tetradecapeptide linker, L2, are inserted between TGF.alpha. and PE38. Together, L2 and CH2 contain 13 ***lysine*** residues as potential modification sites for mPEG, mPEG conjugates of TCP (PEG-TCP) were generated and the products were resolved by ion exchange chromatog. Two PEG-TCP species termed B4 and B6 retained 15 and 4% of cytotoxicity, resp., and 26% of their receptor binding activity compared with the unmodified TCP. Both B4 and B6 had prolonged circulation times in the blood and reduced toxicity in animals. The mean residence times of B4 and B6 were 37 and 68 min, resp., compared to 7 min for TCP. When administered i.v. to tumor bearing mice, both B4 and B6 produced marked antitumor effects whereas the unmodified TCP had none. Also, the immunogenicity of PEG-TCP was 5-10 times less than that of TCP. The authors suggest that the prolonged circulating time and reduced toxicity of ***PEG*** -TCP compensate for a diminished cytotoxic ***activity*** and enlarge significantly the therapeutic window of this chimeric toxin.

L25 ANSWER 15 OF 27 CAPLUS COPYRIGHT 2002 ACS

1994:71885 Document No. 120:71885 Dehydration-induced conformational transitions in proteins and their inhibition by stabilizers. Prestrelski, Steven; Tedeschi, Nicole; Arakawa, Tsutomu; Carpenter, John F. (Dep. Protein Chem., Amgen, Inc., Thousand Oaks, CA, 91320, USA). Biophysical Journal, 65(2), 661-71 (English) 1993. CODEN: BIOJAU. ISSN: 0006-3495.

AB Dehydration of proteins results in significant, measurable conformational changes as obsd. using Fourier-transform IR spectroscopy and resoln.-enhancement techniques. For several proteins these conformational changes are at least partially irreversible, since, upon rehydration, denaturation and aggregation are obsd. The presence of certain stabilizers inhibited these dehydration-induced transitions; the native structure was preserved in the dried state and upon reconstitution. Conformational transitions were also obsd. in a model polypeptide, poly-L-***lysine*** , after lyophilization and were inhibited with the addn. of

stabilizing cosolutes. The ability of a particular additive to preserve the aq. structure of dehydrated proteins and poly-L- ***lysine*** upon dehydration correlates directly with its ability to preserve the ***activity*** of lactate dehydrogenase, a labile enzyme, during drying.

L25 ANSWER 16 OF 27 CAPLUS COPYRIGHT 2002 ACS

1993:598293 Document No. 119:198293 Surface modification of horseradish peroxidase with poly(ethylene glycol)s of various molecular masses. Fortier, Guy; Laliberte, Maryse (Dep. Chim. Biochim., Univ. Quebec, Montreal, QC, HC3 3P8, Can.). Biotechnology and Applied Biochemistry, 17(1), 115-30 (English) 1993. CODEN: BABIEC. ISSN: 0885-4513.

AB Five different poly(ethylene glycol)s (PEG) and 3 different monomethoxypoly(ethylene glycol)s (mPEG), of mol. wts. in the range of 750-35,000, were activated using 4-nitrophenyl chloroformate in acetonitrile in the presence of triethylamine for 5 h at 60.degree.. This was carried out in order to obtain a high yield of PEG-dinitrophenyl carbonates and mPEG-nitrophenyl carbonates, resp. The reaction conditions (ratio of reagents, temp., and pH) for the coupling of activated m/PEG to the free amino group of ***lysine*** were 1st investigated by using .alpha.-carbobenzoxylysine. It was shown that the formation of the urethane bond between the .delta.-NH2 group of ***lysine*** and the activated m/PEG occurred over a wide range of pH and temp. values and at various molar ratios of reagents. With horseradish peroxidase (HRP), the coupling procedure was carried out at pH 9.2 for 12 h at 4.degree.. Under these conditions, 4 out of the 6 NH2 groups of HRP reacted with the different activated PEGs or mPEGs as demonstrated by the trinitrobenzenesulfonate assay. The elimination of p-nitrophenol and excess m/PEG at the end of the reaction was performed by a new ultrafiltration procedure using a soln. of 0.65M K2SO4, pH 6.5, which permitted a lowering of the viscosity of the m/PEG/HRP-PEG soln. and avoided blocking of the ultrafiltration membrane with free m/PEG. When this procedure was followed, a mol. wt. detn. was achieved by HPLC gel filtration in the presence of 8M urea. The pI values of the various prepns. occurred at a lower pH of 7.0 when compared with the native HRP. Furthermore, all of the modified HRP- ***PEG*** prepns. showed high ***activity*** for the following substrates: o-dianisidine, o-phenylenediamine, or aminoantipyrine in the presence of H2O2. No systematic decrease in specific ***activity*** was correlated to the mol. wt. of the m/ ***PEG*** used for derivatization.

L25 ANSWER 17 OF 27 CAPLUS COPYRIGHT 2002 ACS

1993:66642 Document No. 118:66642 The uses and properties of PEG-linked proteins. Delgado, Cristina; Francis, Gillian E.; Fisher, Derek (Sch. Med., R. Free Hosp., London, UK). Critical Reviews in Therapeutic Drug Carrier Systems, 9(3-4), 249-304 (English) 1992. CODEN: CRTSEO. ISSN: 0743-4863.

AB A review with 213 refs. Poly(ethylene glycol) (PEG) is a water sol. polymer that when covalently linked to proteins, alters their properties in ways that extend their potential uses. PEG-modified conjugates are being exploited in many different fields. The improved pharmacol. performance of PEG-proteins when compared with their unmodified counterparts prompted the development of this type of conjugate as a therapeutic agent. Enzyme deficiencies for which therapy with the native enzyme was inefficient (due to rapid clearance and/or immunol. reactions) can now be treated with equiv. PEG-enzymes. PEG-adenosine deaminase has already obtained FDA approval. PEG-modified cytokines have been constructed and, interestingly, one of the conjugates, PEG-modified granulocyte-macrophage colony-stimulating factor, showed dissocn. of two biol. properties. This novel observation may open new horizons to the application of PEGylation technol. The biotechnol. industry has also found PEG-proteins very useful because PEG-enzymes can act as catalysts in org. solvents, thereby opening the possibility of producing desired stereoisomers, as opposed to the racemic mixt. usually obtained in classical org. synthesis. Covalent attachment of PEG to proteins requires activation of the hydroxyl terminal group of the polymer with a suitable leaving group that can be displaced by nucleophilic attack of the .epsilon.-amino terminal of ***lysine*** residues (other nucleophilic groups can also interact). Several chem. groups have been exploited to activate PEG, thereby giving rise to a variety of PEG-proteins. Some of these varieties retain part of the activating group as a coupling moiety between PEG and protein and others provide a direct linkage. For each

particular application, different coupling methods provide distinct advantages. The development of newer methods should increase the no. of biol. active PEG-proteins and, possibly, the no. of applications. Also, a deeper understanding of the mol. basis for the altered physicochem. properties will facilitate the development of designed ***PEG*** -modified proteins with the desired spectrum of biol. ***activities*** and, hence, new applications.

L25 ANSWER 18 OF 27 CAPLUS COPYRIGHT 2002 ACS
1991:119942 Document No. 114:119942 Characterization of a polyethylene glycol conjugate of recombinant human interferon-.gamma.. Kita, Yoshiko; Rohde, Michael F.; Arakawa, Tsutomu; Fagin, Katherine D.; Fish, Eleanor N.; Banerjee, Kris (Amgen Inc., Thousand Oaks, CA, 91320, USA). Drug Design and Delivery, 6(3), 157-67 (English) 1990. CODEN: DDDEEJ. ISSN: 0884-2884.
AB Recombinant human interferon-.gamma. was conjugated with polyethylene glycol (PEG) using succinimidyl coupling of amino groups in the protein. The ***PEG*** conjugated material showed antiviral, growth inhibitory and macrophage activation ***activities*** indistinguishable from those of the unmodified protein. The PEG conjugation reduced the receptor binding affinity slightly, but increased the half-life of the protein when measured in rats. Almost no clearance was obsd. within 6 h after injection for the PEG conjugated protein, whereas a rapid clearance was seen for the unmodified interferon-.gamma.. Two possible sites of PEG attachment were identified in the protein: the N-terminal amino group and either ***lysine*** 129 or 131.

L25 ANSWER 19 OF 27 CAPLUS COPYRIGHT 2002 ACS
1989:22414 Document No. 110:22414 Correlation between induction time and rate of browning in heated model solutions of glucose and ***lysine*** . Petriella, C.; Chirife, J.; Resnik, Silvia L.; Lozano, R. D. (Fac. Cienc. Exactas Nat., Univ. Buenos Aires, Buenos Aires, 1428, Argent.). Int. J. Food Sci. Technol., 23(4), 415-18 (English) 1988. CODEN: IJFTEZ. ISSN: 0950-5423.
AB The effects of polyol humectants and salts on induction period and rate of visual browning of glucose and ***lysine*** were studied at 45, 55, 60, and 65.degree. and water activities of 0.9, 0.925, and 0.95. A high rate of browning was preceded by a short induction period. The type of salt (KCl, NaCl, Na2SO4) influenced browning, but polyol type did not. Temp. and pH (4-6) also had a strong effect on the rate of browning.

L25 ANSWER 20 OF 27 CAPLUS COPYRIGHT 2002 ACS
1988:453361 Document No. 109:53361 Solute effects at high water ***activity*** on nonenzymic browning of glucose- ***lysine*** solutions. Petriella, Claudio; Chirife, Jorge; Resnik, Silvia L.; Lozano, Roberto D. (Fac. Cienc. Exactas Nat., Univ. Buenos Aires, Buenos Aires, 1428, Argent.). J. Food Sci., 53(3), 987-8 (English) 1988. CODEN: JFDSAZ. ISSN: 0022-1147.
AB The effect of different polyols (glycerol, propylene glycol, sorbitol, 1,3-butylene glycol, polyethylene glycol 200, and polyethylene glycol 400) and various alkali metal chlorides (KCl, NaCl, LiCl, and CsCl) on the kinetics of nonenzymic browning in glucose- ***lysine*** solns., of high water ***activity*** (aw) was studied. No particular inhibitory or promoting effect on browning of glucose- ***lysine*** solns. when adjusting aw with the various polyols, was found. The alkali metal chlorides had a significant influence on the rate of browning and the relative effect of cations (Li>Na>K,Cs) paralleled the hydrated ionic radii in solns.

L25 ANSWER 21 OF 27 CAPLUS COPYRIGHT 2002 ACS
1988:2530 Document No. 108:2530 Effects of polyethylene glycol substitution on enzyme activity. Yoshinaga, Kohji; Shafer, Steven G.; Harris, J. Milton (Dep. Chem., Univ. Alabama, Huntsville, AL, 35899, USA). J. Bioact. Compat. Polym., 2(1), 49-56 (English) 1987. CODEN: JBCPEV. ISSN: 0883-9115.
AB The effects of polyethylene glycol ( ***PEG*** ) substitution on protein ***activity*** , using alk. phosphatase as a model, were studied. Such variables as PEG mol. wt., degree of substitution, and PEG mono- and bifunctionality were examd. Modification with the monomethyl ether of ***PEG*** 1900 (M- ***PEG*** -1900) did not alter enzyme ***activity*** until >40% of the protein ***lysine*** groups were

substituted, at which point increasing the degree of modification gave increasing deactivation. Substitution with M-PEG-5000 gaves more deactivation than did substitution with M-PEG-1900. Interestingly, modification with PEG itself gave active protein conjugates in which there was little dependence on mol. wt. or degree of substitution.

L25 ANSWER 22 OF 27 CAPLUS COPYRIGHT 2002 ACS
1986:213114 Document No. 104:213114 Enzyme:polyethylene glycol conjugates in the delivery of enzyme therapy. Davis, Frank F.; Abuchowski, Abraham (Enzon, Inc., South Plainfield, NJ, 07080, USA). Polym. Prepr. (Am. Chem. Soc., Div. Polym. Chem.), 27(1), 5-6 (English) 1986. CODEN: ACPPAY. ISSN: 0032-3934.
AB Results of a no. of years study on polyethylene glycol (PEG)-enzyme conjugates are presented. Enzymes were linked to PEG Me ether by reaction of the PEG with succinic anhydride, N-hydroxysuccinimide and then reaction with the enzyme. The ***PEG*** -enzyme showed >50% ***activity*** in excess of unmodified enzyme. They also exhibited plasma half-lives of 1-2 orders of magnitude greater than unmodified ones. They were also more stable e.g., PEG-asparaginase at pH 7 and 4.degree. shows no loss of activity in 2 yr. PEG-enzymes can be administered by i.v., i.p., i.m. or s.c. routes. In a study on efficacy of PEG-enzymes serum urate levels in humans were decreased to undetectable levels in 2-3 h after injection of PEG- ***uricase*** compared to allopurinol which requires days or weeks to decrease serum urate to normal levels.

L25 ANSWER 23 OF 27 CAPLUS COPYRIGHT 2002 ACS
1985:22261 Document No. 102:22261 Comparison of thrombolytic activity of native and modified urokinase, using an experimental thrombosis model in the dog. Takatsuka, Jun; Kaneko, Hironori; Isogai, Masahiro; Shiba, Tadaaki; Takeuchi, Setsuo; Igarashi, Michiko; Asada, Toshio; Shimizu, Kimihiro; Kondo, Kaname; Ishikura, Akihiro (Sch. Med., Toho Univ., Tokyo, Japan). Ketsueki to Myakkan, 15(4), 446-8 (Japanese) 1984. CODEN: KTMYA3. ISSN: 0386-9717.
AB Urokinase (UK) was modified by a covalent attachment of methoxypolyethylene glycol (PEG) 5,000 to its ***lysine*** residues after activation of PEG with cyanuric chloride. For the evaluation of thrombolytic ***activity*** of ***PEG*** -mediated UK ( ***PEG*** -UK), a novel exptl. thrombosis model was developed in a dog's femoral artery. Using this model, a strong thrombolytic ***activity*** of ***PEG*** -UK was shown. However, native UK could not dissolve the thrombus at a dose of 100,000 IU/dog. The serum FDP level of the PEG-UK-treated dog reached 1280 .mu.g/mL, while that of the native UK-treated dog stayed at 20 .mu.g/mL.

L25 ANSWER 24 OF 27 CAPLUS COPYRIGHT 2002 ACS
1983:435607 Document No. 99:35607 A new procedure for the synthesis of polyethylene glycol-protein adducts; effects on function, receptor recognition, and clearance of superoxide dismutase, lactoferrin, and .alpha.2-macroglobulin. Beauchamp, Charles O.; Gonias, Steven L.; Menapace, David P.; Pizzo, Salvatore V. (Dep. Med., Univ. Michigan, Ann Arbor, MI, 48105, USA). Anal. Biochem., 131(1), 25-33 (English) 1983. CODEN: ANBCA2. ISSN: 0003-2697.
AB A new, simplified technique for the synthesis of PEG derivs. of proteins utilizing 1,1'-carbonyldiimidazole for PEG activation, is described. PEG derivs. of superoxide dismutase, .alpha.2-macroglobulin(I), I-trypsin, and lactoferrin were prepd. and studied. Superoxide dismutase coupled to ***PEG*** preserved 95% of its original ***activity*** while its plasma half-life increased from 3.5 min to .gtoreq.9 h depending on the PEG deriv. studied. ***PEG*** -derivatized I showed decreased protease binding ***activity*** , but ***PEG*** derivs. of preformed I-trypsin demonstrated no loss of ***activity*** . The blood plasma clearance of PEG-I-trypsin was prolonged significantly compared to native I-trypsin, particularly when a high-mol.-wt. PEG was coupled to the protease inhibitor complex. The plasma clearance half-life of lactoferrin was increased 5- to 20-fold by this modification. Trinitrobenzenesulfonic acid titrn. studies demonstrated that .epsilon.-amino groups of ***lysine*** residues are modified by the coupling of carbonyldiimidazole-activated PEG to proteins.

L25 ANSWER 25 OF 27 CAPLUS COPYRIGHT 2002 ACS
1982:176793 Document No. 96:176793 Macromolecular NAD+-derivative of high

coenzyme activity and its application for a model reaction. Okada, H.; Urabe, I.; Muramatsu, M.; Furukawa, S. (Dep. Ferment. Technol., Osaka Univ., Suita, Japan). Adv. Biotechnol., [Proc. Int. Ferment. Symp.], 6th, Meeting Date 1980, Volume 1, 721-7. Editor(s): Moo-Young, Murray; Robinson, Campbell W.; Vezina, Claude. Pergamon: Toronto, Ont. (English) 1981. CODEN: 47GQAB.

GI

/ Structure 3 in file .gra /

AB NAD-N6-[N-(N-acryloylmethoxycarbonyl-5-aminopentyl)propionamide] (I) was synthesized (Muramatsu, M., et al., 1977) and was then copolymd. with acrylamide, methacrylamide, N-acrylolyl-6-aminohexanoic acid, N.epsilon.-acryloyl-L- ***lysine*** , or N-acryloyl-3,6,9-trioxa-1-aminodecane. High cofactor activities were found for I and its NADH deriv. with various dehydrogenases relative to those of free NAD and NADH. Lower but substantial activities with many, but not all, the dehydrogenases were obtained for I copolymd. with the vinyl monomers, and among the copolymers, the highest coenzyme activity was found with I-methacrylamide copolymer. The activities of rabbit muscle lactate dehydrogenase and yeast alc. dehydrogenase decreased with an increase in the molar ratio of NAD to methacrylamide in the I-methacrylamide copolymer, whereas there was very little effect of NAD molar ratio on pig heart malate dehydrogenase or horse liver alc. dehydrogenase. At the highest NAD molar ratio used, I-methacrylamide copolymer inhibited the redn. of free NAD by rabbit muscle lactate dehydrogenase and yeast alc. dehydrogenase. Considering the above results, a new macromol. NAD deriv. with lower NAD d. was designed and prepd. by coupling of N6-(2-carboxyethyl)-NAD with polyethylene glycol (PEG). This ***PEG*** -NAD had a high cofactor ***activity*** with various dehydrogenases. PEG-NAD was then used for a continuous enzyme reactor which contained lactate and alc. dehydrogenase and produced L-lactate from pyruvate. PEG-NAD was stable for >1 wk of operation in the enzyme reactor, but the enzymes (from mammalian cells) themselves were not suitable for long-term operation in the reactor.

L25 ANSWER 26 OF 27 CAPLUS COPYRIGHT 2002 ACS

1981:43310 Document No. 94:43310 Soluble, nonantigenic polyethylene glycol-bound enzymes. Davis, F. F.; Abuchowski, A.; Van Es, T.; Palczuk, N. C.; Savoca, K.; Chen, R. H. L.; Pyatak, P. (Dep. Biochem., Rutgers, State Univ., New Brunswick, NJ, 08903, USA). Polym. Prepr., Am. Chem. Soc., Div. Polym. Chem., 20(1), 357-60 (English) 1979. CODEN: ACPPAY. ISSN: 0032-3934.

AB Enzymes were modified with methoxypolyethylene glycol (PEG) activated by 2,4,6-trichloro-s-triazine and their properties studied. With the exception of phenylalanine ammonia-lyase, the PEG-enzymes were nonimmunogenic. The enzymes varied in size from monomers (trypsin, adenosine deaminase) and a dimer (superoxide dismutase) to tetramers (arginase, catalase, glutaminase-asparaginase from Achromobacter, and ***uricases*** ). ***PEG*** modification tended to decrease the ***activity*** and increase Km values. ***PEG*** -trypsin showed no change in esterolytic ***activity*** but was only able to hydrolyze smaller peptides. PEG-enzymes showed rapidly decreasing electrophoretic mobility with increasing amts. of PEG attached, did not bind to ion-exchange columns, and sedimented more slowly than the native enzymes. Enzymes normally insol. under physiol. conditions were solubilized by PEG attachment. Both PEG-glutaminase-asparaginase and PEG-superoxide dismutase appeared in blood after i.p. injection in mice in higher amts. than the native enzymes, demonstrating their ability to cross membranes. PEG-superoxide dismutase was more effective than unmodified enzyme in suppressing the inflammatory response to cotton twine implanted s.c. in rats. PEG-glutaminase-asparaginase was more effective in the treatment of tumors than unmodified enzyme.

L25 ANSWER 27 OF 27 CAPLUS COPYRIGHT 2002 ACS

1980:543000 Document No. 93:143000 Preparation of a polyethylene glycol:superoxide dismutase adduct, and an examination of its blood circulating life and anti-inflammatory activity. Pyatak, Peter S.; Abuchowski, Abraham; Davis, Frank F. (Dep. Biochem., Rutgers State Univ.,

New Brunswick, NJ, 08903, USA). Res. Commun. Chem. Pathol. Pharmacol., 29(1), 113-27 (English) 1980. CODEN: RCOCB8. ISSN: 0034-5164.
AB Methoxypolyethylene glycol (PEG) [ ***9004-74-4*** ] of 5000 daltons was attached covalently to 19 of the 20 available ***lysine*** residues of bovine erythrocyte superoxide dismutase [9054-89-1]. The adduct, ***PEG*** -speroxide dismutase, has 51% of the enzymic ***activity*** of superoxide dismutase. PEG-superoxide dismutase exhibits a sharply enhanced serum circulating life during repetitive i.v. injections compared to the native enzyme. While no evidence of an immune response to repetitive injections of PEG-superoxide dismutase is obsd., the unmodified enzyme appears to produce slight sensitization in the form of quicker removal from the serum after 13 injections over a period of 30 days. Antisera to superoxide dismutase and PEG-superoxide dismutase, however, produce no detectable antibodies as detd. by the Ouchterlony method. I.p. injected PEG-superoxide dismutase enters the blood stream more readily than superoxide dismutase. ***PEG*** modification slightly improves the enzyme's antiinflammatory ***activity*** , which was obsd. in rats over a period of 8 days.

=> S (L4,L6) (10A) L19
L26 486 ((L4 OR L6)) (10A) L19

=> S L17 AND L26
L27 9 L17 AND L26

=> S L27 NOT L25
L28 6 L27 NOT L25

=> D 1-6 CBIB ABS

L28 ANSWER 1 OF 6 CAPLUS COPYRIGHT 2002 ACS
2000:305995 Correction of: 1997:343423 Document No. 132:292543 Correction of: 127:64330 Positional isomers of monopegylated interferon .alpha.-2a: isolation, characterization, and biological activity. Monkarsh, Seth P.; Ma, Yuemei; Aglione, Anthony; Bailon, Pascal; Ciolek, Doreen; DeBarbieri, Barbara; Graves, Mary C.; Hollfelder, Kurt; Hanspeter, Michel; Palleroni, Alicia; Porter, Jill E.; Russoman, Emil; Roy, Swapan; Pan, Yu-Ching E. (Hoffmann-La Roche Inc., Nutley, NJ, 07110, USA). Analytical Biochemistry, 247(2), 434-440 (English) 1997. CODEN: ANBCA2. ISSN: 0003-2697. Publisher: Academic.
AB The success of recombinant interferon .alpha. in the clinic in part is limited by two properties of the protein: short serum half-life and immunogenicity. To improve these properties, interferon .alpha.-2a was conjugated with polyethylene glycol (PEG-5000). A homogeneous prepn. of monopegylated interferon .alpha.-2a was subjected to vigorous anal. and activity characterization. A newly developed ampholyte-free chromatofocussing-like cation-exchange HPLC method utilizing a sulfopropyl resin was used to sep. the monopegylated protein into 11 species. Peptide mapping, sequencing, and mass spectrometric analyses indicated that these species are positional isomers where each isomer represents a single polymer mol. conjugated to one specific ***lysine*** residue. No species with a modification at the amino terminus was obsd. All 11 isomers show antiviral and antiproliferative activities in the same range as the parent monopegylated interferon .alpha.-2a.

L28 ANSWER 2 OF 6 CAPLUS COPYRIGHT 2002 ACS
1999:113575 Document No. 130:172747 Polymer-modified enzymes with high activity and reduced allergenicity. Weisgerger, David; Rubingh, Donn Nelton (The Procter & Gamble Company, USA). PCT Int. Appl. WO 9906071 A1 19990211, 34 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1998-US15282 19980723. PRIORITY: US 1997-903298 19970730.
AB The present invention relates to a modified polypeptide which has an enzymic activity level of greater than about 70% of the parent polypeptide

and an allergenic response level of less than about 33% of the parent polypeptide. Embodiments of the present invention relate to modified polypeptides with reduced allergenicity and high enzymic activity comprising the formula: A-Bn, wherein A is an enzyme, and mixts. thereof; B is a twin polymer moiety, having a total mol. wt. of from about 0.5 kDa to about 40 kDa, conjugated to the enzyme; wherein R1 and R2 on the polymer moiety are essentially straight chain polymers, having a mol. wt. ranging from about 0.25 to about 20 kDa; wherein the ratio of the mol. wts. of R1 and R2 is from about 1:10 to about 10:1, wherein X is a linking moiety which links the twin moiety to a single site on the enzyme; and n is the no. of twin polymer moieties conjugated to the enzyme, and represents an integer from about 1 to about 15. Thus, protease B (a mutagenized subtilisin BPN') is conjugated with an av. of 3 twin polymer moieties consisting of 2 polyethylene glycol moieties, each with a mol. wt. of 5000 kDa and an activated ***lysine*** succinimidyl ester. The modified enzymes may be used in a variety of personal care products, including skin care compns., shower gels, skin moisturizers, cosmetic compns., and cleansing wipe compns.

L28 ANSWER 3 OF 6 CAPLUS COPYRIGHT 2002 ACS
1997:783625 Document No. 128:47304 Polyethylene glycol conjugates of .alpha.-interferons. Bailon, Pascal Sebastian; Palleroni, Alicia Vallejo (F. Hoffmann-La Roche A.-G., Switz.). Eur. Pat. Appl. EP 809996 A2 19971203, 16 pp. DESIGNATED STATES: R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO. (English). CODEN: EPXXDW. APPLICATION: EP 1997-108261 19970522. PRIORITY: US 1996-18834 19960531.
AB The authors disclose a new class of derivs. of interferon-.alpha. (IFN-.alpha.) produced by attachment of a branched moiety incorporating two linear polyethylene glycol chains. The attachment moiety is derived from mono-methoxy PEG derivatization of ***lysine*** at the .alpha. and .epsilon. amino groups followed by N-hydroxysuccinimide conjugation of IFN-.alpha.. Compared to unmodified IFN-.alpha., these derivs. have an increased half-life in circulation, reduced immunogenicity, decreased clearance, and increased anti-proliferative activity.

L28 ANSWER 4 OF 6 CAPLUS COPYRIGHT 2002 ACS
1997:343423 Document No. 127:64330 Positional isomers of monopegylated interferon .alpha.-2a: isolation, characterization, and biological activity. Monkarsh, Seth P.; Ma, Yuemei; Aglione, Anthony; Bailon, Pascal; Ciolek, Doreen; DeBarbieri, Barabara; Graves, Mary C.; Hollfelder, Kurt; Michel, Hanspecter; Palleroni, Alicia; Porter, Jill E.; Russoman, Emil; Roy Swapan; Pan, Yu-Ching E. (Dep. Biopharmaceuticals, Hoffmann-La Roche Inc., Nutley, NJ, 07110, USA). Analytical Biochemistry, 247(2), 434-440 (English) 1997. CODEN: ANBCA2. ISSN: 0003-2697. Publisher: Academic.
AB The success of recombinant interferon .alpha. in the clinic in part is limited by two properties of the protein: short serum half-life and immunogenicity. To improve these properties, interferon .alpha.-2a was conjugated with polyethylene glycol (PEG-5000). A homogeneous prepn. of monopegylated interferon .alpha.-2a was subjected to vigorous anal. and activity characterization. A newly developed ampholyte-free chromatofocussing-like cation-exchange HPLC method utilizing a sulfopropyl resin was used to sep. the monopegylated protein into 11 species. Peptide mapping, sequencing, and mass spectrometric analyses indicated that these species are positional isomers where each isomer represents a single polymer mol. conjugated to one specific ***lysine*** residue. No species with a modification at the amino terminus was obsd. All 11 isomers show antiviral and antiproliferative activities in the same range as the parent monopegylated interferon .alpha.-2a.

L28 ANSWER 5 OF 6 CAPLUS COPYRIGHT 2002 ACS
1997:75823 Document No. 126:168293 Desensitization to glucose 6-phosphate of phosphoenolpyruvate carboxylase from maize leaves by pyridoxal 5'-phosphate. Tovar-Mendez, Alejandro; Mujica-Jimenez, Carlos; Munoz-Clares, Rosario A. (Departamento de Bioquimica, Facultad de Quimica, Universidad Nacional Autonoma de Mexico, Mexico D.F., 04510, Mex.). Biochimica et Biophysica Acta, 1337(2), 207-216 (English) 1997. CODEN: BBACAQ. ISSN: 0006-3002. Publisher: Elsevier.
AB Incubation of the nonphosphorylated form of maize-leaf phosphoenolpyruvate carboxylase (orthophosphate: oxaloacetate carboxy-lyase (phosphorylating),

PEPC, EC 4.1.1.31) with the reagent pyridoxal 5'-phosphate (PLP) resulted in time-dependent, reversible inactivation and desensitization to the activator glucose 6-phosphate (Glc6P) and other related phosphorylated compds. Both processes are not connected, since (i) when the PLP-modification was carried out in the presence of satg. ligands of the active site, which prevents inactivation, the desensitization to Glc6P is still obsd., and (ii) under some exptl. conditions the desensitization reaction is 4-times faster than the inactivation. Desensitization to Glc6P is first order with respect to PLP and has a second-order forward rate const. of 4.7.+-.0.3 s-1 M-1 and a first-order reverse rate const. of 0.0046.+-.0.0002 s-1. Correlation studies between the remaining Glc6P sensitivity and mol of PLP residues incorporated per mol of enzyme subunit indicate that one lysyl group per enzyme monomer is involved in the sensitivity of the enzyme to Glc6P. The reactivity of this group is increased by polyethylene glycol and glycerol, while the reactivity of the lysyl group of the active site is not affected by these org. cosolutes. In the presence, but not in the absence, of the org. cosolutes, Glc6P by itself offers significant protection against desensitization, while increasing the extent of inactivation. Free PEP or PEP-Mg have opposite effects, protecting the enzyme against inactivation and increasing the degree of desensitization. They also increase the protection against desensitization afforded by Glc6P. Finally, the PEPC inhibitor malate provides some protection against both inactivation and desensitization. Taken together, these results are consistent with PLP-modification of a highly reactive lysyl group at or near the allosteric Glc6P-site.

L28 ANSWER 6 OF 6 CAPLUS COPYRIGHT 2002 ACS
1979:489320 Document No. 91:89320 Immunological properties of protein conjugates with non-immunogenic polymers: studies with ragweed pollen allergen, antigen E. King, Te Piao (Rockefeller Univ., New York, NY, USA). Versatility Proteins, [Proc. Int. Symp. Proteins], 335-51. Editor(s): Li, Choh Hao. Academic: New York, N. Y. (English) 1978. CODEN: 40WBAX.
AB Conjugates of ragweed allergen E coupled to methoxypolyethylene glycol (PEG) via cyanuric chloride, or to the random copolymer of D-glutamic acid and D- ***lysine*** (D-GL) via intermol. disulfide bond formation, had reduced antigenic, allergenic, and immunogenic properties, but retained the immunosuppressive properties of native antigen. Apparently, allergenicity and immunogenicity of a protein depends upon multivalency of the antigen, whereas immunosuppression does not.

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=> E HERSHFIELD M/AU
=> S E7,E8
             5 "HERSHFIELD MICHAEL"/AU
            84 "HERSHFIELD MICHAEL S"/AU
L29         89 ("HERSHFIELD MICHAEL"/AU OR "HERSHFIELD MICHAEL S"/AU)

=> E KELLY S/AU
=> S E3,E11,E122-E124
           100 "KELLY S"/AU
            12 "KELLY S J"/AU
            16 "KELLY SUSAN J"/AU
             1 "KELLY SUSAN JEAN S"/AU
             1 "KELLY SUSAN JELINEK"/AU
L30        130 ("KELLY S"/AU OR "KELLY S J"/AU OR "KELLY SUSAN J"/AU OR "KELLY
               SUSAN JEAN S"/AU OR "KELLY SUSAN JELINEK"/AU)

=> E HERSHFIELD M/AU
=> S E4,E7,E8
             4 "HERSHFIELD M S"/AU
             5 "HERSHFIELD MICHAEL"/AU
            84 "HERSHFIELD MICHAEL S"/AU
L31         93 ("HERSHFIELD M S"/AU OR "HERSHFIELD MICHAEL"/AU OR "HERSHFIELD
               MICHAEL S"/AU)

=> S L30,L31
L32        219 (L30 OR L31)

=> S L32 AND (L4,L6,L7)
L33         11 L32 AND ((L4 OR L6 OR L7))
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=> S L33 NOT (L25,L28)
L34 11 L33 NOT ((L25 OR L28))

=> D 1-11 CBIB ABS

L34 ANSWER 1 OF 11 CAPLUS COPYRIGHT 2002 ACS
2001:384369 Document No. 136:79541 Diabetes insipidus in uricase-deficient mice: A model for evaluating therapy with poly(ethylene glycol)-modified uricase. ***Kelly, Susan J.*** ; Delnomdedieu, Marielle; Oliverio, Michael I.; Williams, L. David; Saifer, Mark G. P.; Sherman, Merry R.; Coffman, Thomas M.; Johnson, G. Allan; ***Hershfield, Michael S.*** (Divisions of Rheumatology, Department of Medicine, Duke University School of Medicine, Durham, NC, USA). Journal of the American Society of Nephrology, 12(5), 1001-1009 (English) 2001. CODEN: JASNEU. ISSN: 1046-6673. Publisher: Lippincott Williams & Wilkins.
AB Uricase-deficient mice develop uric acid nephropathy, with high mortality rates before weaning. Urate excretion was quantitated and renal function was better defined in this study, to facilitate the use of these mice as a model for evaluating poly(ethylene glycol)-modified recombinant mammalian uricases ( ***PEG*** -uricase) as a potential therapy for gout and uric acid nephropathy. The uric acid/creatinine ratio in the urine of uricase-deficient mice ranges from 10 to >30; on a wt. basis, these mice excrete 20- to 40-fold more urate than do human subjects. These mice consistently develop a severe defect in renal concg. ability, resulting in an approx. sixfold greater urine vol. and a fivefold greater fluid requirement, compared with normal mice. This nephrogenic diabetes insipidus leads to dehydration and death of nursing mice but, with adequate water replacement, high urine flow protects adults from progressive renal damage. Treatment of uricase-deficient mice with ***PEG*** -uricase markedly reduced urate levels and, when initiated before weaning, preserved the renal architecture (as evaluated by magnetic resonance microscopy) and prevented the loss of renal concg. function. ***PEG*** -uricase was far more effective and less immunogenic than unmodified uricase. Retention of uricase in most mammals and its loss in humans and some other primates may reflect the evolution of renal function under different environmental conditions. ***PEG*** -uricase could provide an effective therapy for uric acid nephropathy and refractory gout in human patients.

L34 ANSWER 2 OF 11 CAPLUS COPYRIGHT 2002 ACS
2000:117191 Document No. 132:148491 Urate oxidases of pig and baboon and the genes encoding them and the development of serum-stable non-immunogenic enzymes for the therapeutic breakdown of uric acid. ***Hershfield,*** *** Michael*** ; ***Kelly, Susan J.*** (Duke University, USA). PCT Int. Appl. WO 2000008196 A2 20000217, 69 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US17678 19990805. PRIORITY: US 1998-95489 19980806.
AB Uricases that show sequence similarities to the uricase found at extremely low levels in the human liver are identified in pig and baboon and cDNAs encoding them are cloned for treatment of hyperuricemia and hyperuricosuria, e.g. gout and as a complication of leukemia. The proteins may be used to create novel uricases, such as fusion proteins, with low immunogenicity or improved serum stability and bioavailability. CDNAs were cloned from liver by std. RT-PCR methods and two fusion proteins with N-terminal regions from the pig enzyme and C-terminal regions from the baboon enzyme were constructed by std. methods. The fusion proteins showed .gtoreq.5-fold higher specific activity than the baboon enzyme and .gtoreq.1.2-fold higher specific activity than the pig enzyme. The PEGylated form of one of the fusion proteins retained 62% of its activity with near normal kinetic properties. In mice, the PEGylated enzyme had a circulating half-life of about 48 h compared to <2 h for the unmodified enzyme.

L34 ANSWER 3 OF 11 CAPLUS COPYRIGHT 2002 ACS
2000:116930 Document No. 132:185401 ***PEG*** -urate oxidase conjugates

and use thereof. Williams, L. David; ***Hershfield, Michael S.*** ; ***Kelly, Susan J.*** ; Saifer, Mark G. P.; Sherman, Merry R. (Mountain View Pharmaceuticals, Inc., USA; Duke University). PCT Int. Appl. WO 2000007629 A2 20000217, 53 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ, DE, DE, DK, DK, EE, EE, ES, FI, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US17514 19990802. PRIORITY: US 1998-130392 19980806.

AB A naturally occurring or recombinant urate oxidase (uricase) covalently coupled to poly(ethylene glycol) or poly(ethylene oxide) (both referred to as ***PEG*** ), wherein an av. of 2 to 10 strands of ***PEG*** are conjugated to each uricase subunit and the ***PEG*** has an av. mol. wt. between about 5 kDa and 100 kDa. The resulting ***PEG*** -uricase conjugates are substantially non-immunogenic and retain at least 75 % of the uricolytic activity of the unmodified enzyme.

L34 ANSWER 4 OF 11 CAPLUS COPYRIGHT 2002 ACS

1998:710395 Document No. 130:108270 Adenosine deaminase deficiency: clinical expression, molecular basis, and therapy. ***Hershfield, Michael S.*** (Departments of Medicine and Biochemistry, Duke University Medical Center, Durham, NC, 27710, USA). Seminars in Hematology, 35(4), 291-298 (English) 1998. CODEN: SEHEA3. ISSN: 0037-1963. Publisher: W. B. Saunders Co..

AB A review. with 75 refs. Adenosine deaminase (ADA) deficiency is the first known cause of severe combined immunodeficiency disease (SCID). Over the past 25 yr, the metabolic basis for immune deficiency has largely been established. The clin. spectrum assocd. with ADA deficiency is now quite broad, including older children and adults. The ADA gene has been sequenced, the structure of the enzyme has been detd., and over 50 ADA gene mutations have been identified. There appears to be a quant. relationship between residual ADA activity, detd. by genotype, and both metabolic and clin. phenotype. ADA deficiency has become a focus for novel approaches to enzyme replacement and gene therapy. Enzyme replacement with polyethylene glycol ( ***PEG*** )-modified ADA, used to treat patients who lack a human leukocyte antigen (HLA)-matched bone marrow donor, is safe and effective, but expensive. Several approaches to gene therapy have been investigated in patients receiving ***PEG*** -ADA. Persistent expression of transduced ADA cDNA in T lymphocytes and myeloid cells has occurred in a few patients, but significant improvement in immune function because of the transduced cells has not been shown. The major barrier to effective gene therapy remains the low efficiency of stem cell transduction with retroviral vectors.

L34 ANSWER 5 OF 11 CAPLUS COPYRIGHT 2002 ACS

1997:758010 Document No. 128:99168 Biochemistry and immunology of poly(ethylene glycol)-modified adenosine deaminase ( ***PEG*** -ADA). ***Hershfield, Michael S.*** (Dep. Med. Biochem., Duke Univ. Med. Cent., Durham, NC, 27710, USA). ACS Symposium Series, 680(Poly(ethylene glycol)), 145-154 (English) 1997. CODEN: ACSMC8. ISSN: 0097-6156. Publisher: American Chemical Society.

AB A review with 23 refs. Poly(ethylene glycol)-modified bovine adenosine deaminase ( ***PEG*** -ADA) was the first PEGylated protein to undergo clin. trial. It has been effective in correcting the toxic biochem. effects of ADA substrates, and in treating the fatal immune deficiency disease caused by ADA deficiency. The experience gained from monitoring ***PEG*** -ADA therapy over the past decade provides unique insight into the immunol. response to chronic treatment with a PEGylated enzyme.

L34 ANSWER 6 OF 11 CAPLUS COPYRIGHT 2002 ACS

1997:224405 Document No. 126:271980 Biochemistry and immunology of poly(ethylene glycol)-modified adenosine deaminase ( ***PEG*** -ADA). ***Hershfield, Michael S.*** (Dep. Medicine Biochem., Duke Univ. Med. Center, Durham, NC, 27710, USA). Polymer Preprints (American Chemical Society, Division of Polymer Chemistry), 38(1), 567 (English) 1997. CODEN: ACPPAY. ISSN: 0032-3934. Publisher: American Chemical Society, Division of Polymer Chemistry.

AB Given once or twice a week, at total weekly doses ranging from 15 to 60 Units/kg, ***PEG*** -ADA therapy maintains trough (pre-injection)

plasma ADA activity at about 2-10 times the normal level of total blood ADA activity. It is not detectably taken up by erythrocytes, but by eliminating circulating dAdo., it normalizes levels of dAdo nucleotides and S-adenosylhomocysteine hydrolase (AdoHcyase) activity in these cells. Following a lag of several weeks to a few months, required for maturation of lymphocytes from progenitor cells, immune function improves in most patients to a level that, although not normal, is sufficient to sustain good health. Roughly, coincident with return of immune function, the majority of patients develop antibody to bovine ADA, detectable by ELISA; however, there have been no allergic or other adverse reactions, and clearing or blocking antibodies have developed in only a few cases.

L34 ANSWER 7 OF 11 CAPLUS COPYRIGHT 2002 ACS

1997:164109 Biochemistry and immunology of polyethylene glycol-modified adenosine deaminase ( ***PEG*** -ADA).. ***Hershfield, M. S.*** (Departments Medicine and Biochemistry, Duke University Medical Center, Durham, NC, 27710, USA). Book of Abstracts, 213th ACS National Meeting, San Francisco, April 13-17, POLY-184. American Chemical Society: Washington, D. C. (English) 1997. CODEN: 64AOAA.

AB The first therapeutic trial of a PEGylated enzyme began in 1986, when we used i.m. bovine adenosine deaminase (ADA) conjugated with 5 kDa ***PEG*** , manufd. by Enzon, Inc., to treat severe combined immunodeficiency due to inherited ADA deficiency (Hershfield et al, N Engl J Med 316:589, 1987). During the past decade ***PEG*** -ADA, injected once or twice weekly, has been used in over 60 patients with this rare, fatal disorder; 40 have been treated for >2 yr (mean 5.5 yr). We have monitored biochem. parameters in 50 of these patients. ***PEG*** -ADA therapy maintains high plasma ADA activity, effectively eliminating toxic ADA substrates as indicated by correction of metabolic abnormalities in erythrocytes. Treatment restores immune function to a level sufficient to sustain good health. The majority of patients develop antibody to ***PEG*** -ADA, but there have been no allergic or other adverse reactions, and clearing or blocking antibody has developed in only a few cases. In 1990, ***PEG*** -ADA (ADAGEN) became the first parenteral enzyme to be approved by the US Food and Drug Administration for replacement therapy in an inherited metabolic disease. These results suggest that ***PEG*** -modified enzymes can be used safely and effectively for long-term therapy.

L34 ANSWER 8 OF 11 CAPLUS COPYRIGHT 2002 ACS

1995:917423 Document No. 124:21544 ***PEG*** -ADA replacement therapy for adenosine deaminase deficiency: An update after 8.5 years. ***Hershfield, Michael S.*** (Medical Center, Duke University, Durham, NC, 27707, USA). Clinical Immunology and Immunopathology, 76(3, Pt. 2), S228-S232 (English) 1995. CODEN: CLIIAT. ISSN: 0090-1229. Publisher: Academic.

AB Polyethylene glycol-modified adenosine deaminase ( ***PEG*** -ADA) has now been used for 8.5 yr as enzyme replacement therapy for immunodeficiency due to ADA deficiency. ***PEG*** -ADA restores a metabolic environment necessary for recovery of immune function. In most cases, the level of function achieved has been sufficient to protect against opportunistic and life-threatening infections. To date, mortality and morbidity with ***PEG*** -ADA have been less than for haploidentical bone marrow transplantation. As a true "orphan drug" used to treat a very small patient population, the cost per patient of ***PEG*** -ADA is very high, but it has been well tolerated, free of adverse reactions, and effective as an alternative for patients who lack an HLA-identical marrow donor, but are considered too ill to undergo haploidentical marrow transplantation. Concomitant treatment with ***PEG*** -ADA has also permitted investigation of gene therapy to be carried out safely.

L34 ANSWER 9 OF 11 CAPLUS COPYRIGHT 2002 ACS

1994:94503 Document No. 120:94503 The role of polyethylene glycol-adenosine deaminase in the evolution of therapy for adenosine deaminase deficiency. ***Hershfield, Michael S.*** (Med. Cent., Duke Univ., Durham, NC, USA). New Concepts Immunodefic. Dis., 417-26. Editor(s): Gupta, Sudhir; Griscelli, Claude. Wiley: Chichester, UK. (English) 1993. CODEN: 59OMAU.

AB A review with 32 refs.

L34 ANSWER 10 OF 11 CAPLUS COPYRIGHT 2002 ACS

1994:23215 Document No. 120:23215 T lymphocyte ontogeny in adenosine deaminase-deficient severe combined immune deficiency after treatment with polyethylene glycol-modified adenosine deaminase. Weinberg, Kenneth; ***Hershfield, Michael S.*** ; Bastian, John; Kohn, Donald; Sender, Leonard; Parkman, Robertson; Lenarsky, Carl (Sch. Med., Univ. South. California, Los Angeles, CA, 90027, USA). Journal of Clinical Investigation, 92(2), 596-602 (English) 1993. CODEN: JCINAO. ISSN: 0021-9738.

AB Adenosine deaminase (ADA) deficiency causes severe combined immune deficiency (SCID) by interfering with the metab. of deoxyadenosine, which is toxic to T lymphocytes at all stages of differentiation. Enzyme replacement with polyethylene glycol-modified ADA ( ***PEG*** -ADA) has been previously shown to correct deoxyadenosine metab. and improve mitogen-induced T lymphocyte proliferation. The authors studied the biochem. and immunol. effects of ***PEG*** -ADA in two infants with ADA-deficient SCID. While in a catabolic state, higher doses of ***PEG*** -ADA than previously described were required to normalize deoxyadenosine metab. After biochem. improvement, the patients recovered immune function in a pattern similar to that obsd. in normal thymic ontogeny and in patients with immunol. reconstitution after bone marrow transplantation. Immune reconstitution was marked by the sequential appearance in the peripheral blood of phenotypic T lymphocytes corresponding to successive stages of thymic differentiation. Functional reconstitution was marked by the successive appearance of mitogen responses dependent on exogenous in vitro IL-2, mitogen responses not requiring exogenous IL-2, antigen-specific responses dependent on exogenous IL-2, and finally, antigen-specific responses not requiring exogenous IL-2. Natural killer function was tested in one patient and normalized with ***PEG*** -ADA therapy. Optimal ***PEG*** -ADA therapy appears to normalize thymic differentiation in ADA-deficient SCID, resulting in normal antigen-specific immune function.

L34 ANSWER 11 OF 11 CAPLUS COPYRIGHT 2002 ACS

1991:550137 Document No. 115:150137 Use of site-directed mutagenesis to enhance the epitope-shielding effect of covalent modification of proteins with polyethylene glycol. ***Hershfield, Michael S.*** ; Chaffee, Sara; Koro-Johnson, Lillian; Mary, Ann; Smith, Albert A.; Short, Steven A. (Med. Cent., Duke Univ., Durham, NC, 27710, USA). Proceedings of the National Academy of Sciences of the United States of America, 88(16), 7185-9 (English) 1991. CODEN: PNASA6. ISSN: 0027-8424.

AB Modification by covalent attachment of polyethylene glycol ( ***PEG*** ) can reduce the immunogenicity and prolong the circulating life of proteins, but the utility of this approach for any protein is restricted by the no. and distribution of ***PEG*** attachment sites (e.g., .epsilon.-amino groups of lysine residues). A strategy for introducing addnl. sites for ***PEG*** attachment was developed by using site-directed mutagenesis to selectively replace arginine with lysine codons; it was tested with purine nucleoside phosphorylase (PNP) from Escherichia coli, an extremely stable but immunogenic enzyme that could potentially be used to treat an inherited deficiency of PNP. A triple mutant, RK3, possessing three Arg .fwdarw. Lys substitutions was constructed that increased the no. of lysines per PNP subunit from 14 to 17, providing an addnl. 18 potential ***PEG*** attachment sites per hexameric enzyme mol. The wild-type and RK3 enzymes had similar catalytic activity, antigenicity, and immunogenicity. After ***PEG*** modification, both enzymes in mice increased from .apprxeq. 4 h to 4 days, and the binding of both enzymes by antisera raised against each unmodified enzyme was markedly diminished. However, antibody raised against wild-type ***PEG*** -PNP did not bind the ***PEG*** -RK3 enzyme. ***PEG*** -RK3 PNP was also substantially less immunogenic than wild-type ***PEG*** -PNP. Accelerated antibody-mediated clearance of ***PEG*** -PNP occurred in 2 of 12 mice treated with ***PEG*** -RK3 PNP, compared with 10 of 16 mice treated with the modified will-type enzyme. This combined use of directed mutagenesis and ***PEG*** modification is aimed at permitting the widest choice of proteins, including products of genetic and chem. "engineering," to be used for therapy of inherited and acquired disorders.